

Amendments to the Specification:

At page 12, delete paragraphs 0030 to 0041, and replace with the following amended paragraphs:

- [0030] **Figure 4 shows *S. Cerevisiae* Alg3 Sequence Comparisons (Blast)**
 Seq. ID No. 24 *S. Cerevisiae* (Query 1)
 Seq. ID No. 25 *S. Cerevisiae* (Subject 1)
 Seq. ID No. 26 *S. Cerevisiae* (Query)
 Seq. ID No. 27 *H. sapiens* (Subject)
 Seq. ID No. 28 *S. Cerevisiae* (Query 1)
 Seq. ID No. 29 *Drosophila virilis* (Subject)
 Seq. ID No. 30 *S. Cerevisiae* (Query)
 Seq. ID. No. 31 *Drosophila melanogaster* (Subject)
- [0031] **Figure 5 shows *S. Cerevisiae* Alg 3 and Alg 3p Sequences**
 Seq. ID No. 32 DNA sequence
 Seq. ID No. 33 amino acid sequence
- [0032] **Figure 6 shows *P. Pastoris* Alg 3 and Alg 3p Sequences**
 Seq. ID No. 34 DNA sequence
 Seq. ID No. 35 amino acid sequence
- [0033] **Figure 7 shows *P. Pastoris* Alg 3 and Alg 3p Sequence Comparisons (Blast)**
 Seq. ID No. 36 *Pichia Pastoris* (Query)
 Seq. ID No. 37 *S. Cerevisiae* (Subject)
 Seq. ID No. 38 (Query)
 Seq. ID No. 39 *Neurospora Crassa* (Subject)
 Seq. ID No. 40 *Pichia Patoris* (Query)
 Seq. ID No. 41 *Schizosaccharomyces pombe* (Subject)
 Seq. ID No. 42 *Pichia Pastoris*
 Seq. ID No. 43 *Arabidopsis thaliana*
- [0034] **Figure 8 shows *K. lactis* Alg 3 and Alg 3p Sequences**
 Seq. ID No. 44 DNA sequence
 Seq. ID No. 45 amino acid sequence

- [0035] **Figure 9 shows *K.lactis* Alg-3 Sequence Comparisons (Blast)**
Seq. ID No. 46 *K. lactis*
Seq. ID No. 47 *S. Cerevisiae*
Seq. ID No. 48 *K. lactis*
Seq. ID No. 49 *Arabidopsis thaliana*
- [0036] **Figure 10 shows *S. Cerevisiae* Alg9 and Alg 9p Sequences**
Seq. ID No. 50 *S. Cerevisiae* Alg 9 DNA
Seq. ID No. 51 *S. Cerevisiae* amino acid
- [0037] **Figure 11 shows *P. Pastoris* Alg-9 and Alg-9p Sequences**
Seq. ID No. 52 *Pichia Pastoris* Alg 9 DNA
Seq. ID No. 53 *Pichia Pastoris* amino acid
- [0038] **Figure 12 shows *P. Pastoris* Alg-9 Sequence Comparisons (Blast)**
Seq. ID No. 54 *Pichia Pastoris* (Query)
Seq. ID No. 55 *S. Cerevisiae* (Subject)
Seq. ID No. 56 *Pichia Pastoris* (Query)
Seq. ID No. 57 *Anopheles gambiae* (Subject)
Seq. ID No. 58 *Pichia Pastoris* (Query)
Seq. ID No. 59 *S. pombe* (Subject)
Seq. ID No. 60 *Pichia Pastoris* (Query)
Seq. ID No. 61 *M. Musculus* (Subject)
Seq. ID No. 62 *Pichia Pastoris* (Query)
Seq. ID No. 63 *H. Sapiens* (Subject)
- [0039] **Figure 13 shows *S. Cerevisiae* Alg-12 and Alg-12p Sequences**
Seq. ID No. 64 *S. Cerevisiae* Alg 12 DNA
Seq. ID No. 65 *S. Cerevisiae* Alg 12 amino acid
- [0040] **Figure 14 shows *P. Pastoris* Alg-12 and Alg-12p Sequences**
Seq. ID No. 66 *Pichia Pastoris* Alg 12 DNA
Seq. ID No. 67 *S. Cerevisiae* Alg 12 amino acid
- [0041] **Figure 15 shows *P. Pastoris* Alg-12 Sequence Comparisons**
Seq. ID No. 68 *Pichia Pastoris* (Query)
Seq. ID No. 69 *S. Cerevisiae* (Subject)
Seq. ID No. 70 *Pichia Pastoris* (Query)
Seq. ID No. 71 *S. pombe* (Subject)
Seq. ID No. 72 *Pichia Pastoris* (Query)
Seq. ID No. 73 *S. pombe* (Subject) --

At page 13, delete paragraphs 0051 and 0052, and replace with the following amended paragraphs:

- [0051] **Figure 25 shows *S. Cerevisiae* Alg6 and Alg 6p Sequences**
Seq. ID No. 74 *S. Cerevisiae* DNA Alg 6
Seq. ID No. 75 *S. Cerevisiae* amino acid
Seq. ID No. 76 *Pichia Pastoris* DNA Alg 6
Seq. ID No. 77 *Pichia Pastoris* amino acid Alg 6
- [0052] **Figure 26 shows *P. Pastoris* Alg 6 and Alg 6p Sequences**
Seq. ID No. 78 *Pichia Pastoris* (Query)
Seq. ID No. 79 *S. Cerevisiae* (Subject)
Seq. ID No. 80 *Pichia Pastoris* (Query)
Seq. ID No. 81 *S. pombe* (Subject)
Seq. ID No. 82 *Pichia Pastoris* (Query)
Seq. ID No. 83 *D. melanogaster* (Subject)
Seq. ID No. 84 *Pichia Pastoris* (Query)
Seq. ID No. 85 *A. thaliana* (Subject) --

At page 14, delete paragraphs 0054 and 0055, and replace with the following amended paragraphs:

- [0054] **Figure 28 shows *K. lactis* Alg 6 and Alg 6p Sequences**
Seq. ID No. 86 *K. lactis* Alg 6 DNA
Seq. ID No. 87 *K. lactis* Alg 6 amino acid
- [0055] **Figure 29 shows *K. lactis* Alg 6 Sequence Comparisons**
Seq. ID No. 88 *K. lactis* Alg 6 DNA
Seq. ID No. 89 *S. Cerevisiae* (Subject)
Seq. ID No. 90 *K. lactis* (Query)
Seq. ID No. 91 *S. pombe* (Subject)
Seq. ID No. 92 *K. lactis* (Query)
Seq. ID No. 93 *A. thaliana* (Subject)
Seq. ID No. 94 *K. lactis* (Query)
Seq. ID No. 95 *H. Sapiens* (Subject) --

At page 14, delete paragraphs 0058 to 0060, and replace with the following amended paragraphs:

- [0058] **Figure 32 shows *M.musculus* GnTHH Nucleic Acid And Amino Acid Sequences**
Seq. ID No. 96 *M. musculus* DNA GnTHH
Seq. ID No. 97 *M. musculus* amino acid GnTHH
- [0059] **Figure 33 shows *H. Sapiens* GnTHV Nucleic Acid And Amino Acid Sequences**
Seq. ID No. 98 *H. Sapiens* DNA GnTIV
Seq. ID No. 99 *H. Sapiens* aa GnTIV
- [0060] **Figure 34 shows *M.musculus* GnTV Nucleic Acid And Amino Acid Sequences**
Seq. ID No. 100 *M. musculus* DNA GnTV
Seq. ID No. 101 *M. musculus* aa GnTV --

At pages 53-54, delete paragraphs 0173 and 0174, and replace with the following amended paragraphs:

--Degenerate primers were generated based on an alignment of Alg3 protein sequences from *S. cerevisiae*, *H. sapiens*, and *D. melanogaster* and were used to amplify an 83 bp product from *P. pastoris* genomic DNA: 5'-GGTGTTTTGTCTTTCTAGATCTTTGCAYTAYCARTT-3' (SEQ ID NO. 1) and 5'-AGAATTTGGTGGGTGAAGAATTCCA- RCACCAYTCRTG-3' (SEQ ID NO. 2). The resulting PCR product was cloned into the pCR2.1 vector (Invitrogen, Carlsbad, Calif.) and sequence analysis revealed homology to known *ALG3/RHK1/NOT56* homologs (Genbank NC.sub.--001134.2, AF309689, NC.sub.--003424.1). Subsequently, 1929 bp upstream and 2738 bp downstream of the initial PCR product were amplified from a *P. pastoris* genomic DNA library (Boehm, T. Yeast May 1999;15(7):563-72) using the internal oligonucleotides 5'-CCTAAGCTGGTATGCGTTCTCTTTGCCATATC-3' (SEQ ID NO. 3) and 5'-GCGGCATAAACAAATAGATGCTATAAAG-3' (SEQ ID NO. 4) along with T3 (5'-AATTAACCCCTCACTAAAGGG-3') (SEQ ID NO. 5) and T7 (5'-GTAA TACGACTCACTATAGGGC-3') (SEQ ID NO. 6) (Integrated DNA Technologies, Coralville, Iowa) in the backbone of the library bearing plasmid lambda ZAP II (Stratagene, La Jolla, Calif.). The resulting fragments were cloned into the pCR2.1-TOPO vector (Invitrogen) and sequenced. From this sequence, a 1395 bp ORF was identified that encodes a protein with 35% identity and 53% similarity to the *S. cerevisiae* ALG3 gene (using BLAST programs). The gene was named PpALG3.

The sequence of *PpALG3* was used to create a set of primers to generate a deletion construct of the *PpALG3* gene by PCR overlap (Davidson et al, 2002 Microbiol. 148(Pt 8):2607-15). Primers below were used to amplify 1 kb regions 5' and 3' of the *PpALG3* ORF and the KAN^R gene, respectively:

4 RCD142 (5'-CCACATCATCCGTGCTACATATAG-3') (SEQ ID NO. 7), RCD144 (5'-ACGAGGCAAGCTAAACAGATCTCGAAGTATCGAGGG TTATCCAG-3') (SEQ ID NO. 8), RCD145 (5'-CCATCCAGTGTGCAAAACGAGC- CAATGGTTTCATGTC TATAAATC-3') (SEQ ID NO. 9), RCD147 (5'-AGCCTCAGCGCCAACAAGCGATGG-3') (SEQ ID NO. 10), RCD143 (5'-CTGGATAACCCTCGATACTTCGAGATCTGTTTAGCT TGCCTCGT-3') (SEQ ID NO. 11), and RCD146 (5'-GATTTATAGACATGAACCATTTGGCTCGTTTC- GACA CTGGATGG-3') (SEQ ID NO. 12)--

At page 55, delete paragraph 0175, and replace with the following amended paragraph:

--The *ALG3p* sequences from *S. cerevisiae*, *Drosophila melanogaster*, *Homo sapiens* etc were aligned with *K. lactis* sequences (PENDANT EST database). Regions of high homology that were in common homologs but distinct in exact sequence from the homologs were used to create pairs of degenerate primers that were directed against genomic DNA from the *K. lactis* strain MG 1/2 (Bianchi et al, 1987). In the case of *ALG3*, PCR amplification with primers KAL-1 (5'-ATCCTTTACCGATGCTAT-3') (SEQ ID NO. 13) and KAL-2 (5'-ATAACAGTATGTGTTACACGCGTGTAG-3') (SEQ ID NO. 14) resulted in a product that was cloned and sequenced and the predicted translation was shown to have a high degree of homology to Alg3p proteins (>50% to *S. cerevisiae* Alg3p).--

At pages 65-66 , delete paragraph 0206, and replace with the following amended paragraph:

--The C₁2 portion harbors a conserved N-glycosylation site at asparagine 297 (Asp297). The Asp297 N-glycans are highly heterogeneous and are known to affect Fc receptor binding and complement activation. Only a minority (i.e., about 15-20%) of IgGs bears a disialylated, and 3-10% have a monosialylated N-glycan (reviewed in Jefferis, R., Glycosylation of human IgG Antibodies. BioPharm, 2001). Interestingly, the minimal N-glycan structure shown to be necessary for fully functional antibodies capable of complement activation and Fc receptor binding is a pentasaccharide with terminal N-acetylglucosamine residues

(GlcNAc.sub.2Man.sub.3) (reviewed in Jeffëris, R., Glycosylation of human IgG Antibodies. BioPharm, 2001). Antibodies with less than a GlcNAc.sub.2Man.sub.3 N-glycan or no N-glycosylation at Asp297 might still be able to bind an antigen but most likely will not activate the crucial downstream events such as phagocytosis and complement activation. In addition, antibodies with fungal-type N-glycans attached to Asp297 will in all likelihood solicit an immune-response in a mammalian organism which will render that antibody useless as a therapeutic glycoprotein.

B. Cloning and Expression of GnTIII

The DNA fragment encoding part of the mouse GnTIII protein lacking the TM domain is PCR amplified from murine (or other mammalian) genomic DNA using forward 5'-TCCTGGCGCGCCTTCCCGAGAGAACTGGCCTCCCTC-3' (SEQ ID NO. 15) and 5'-AATTAATTAACCCCTAGCCCTCCGCTGTATCCAACCTTG-3' (SEQ ID NO. 16) reversed primers. Those primers include AseI and PaeI restriction sites that will be used for cloning into the vector suitable for the fusion with leader library. The nucleic acid and amino acid sequence of murine GnTIII is shown in Fig. 32.--

At page 68, delete paragraphs 0212-0213, and replace with the following amended paragraphs:

--GnTIV-encoding cDNAs were isolated from bovine and human cells (Minowa, M. T. et al. (1998) *J. Biol. Chem.* 273 (19), 11556-11562; and Yoshida, A. et al. (1999) *Glycobiology* 9 (3), 303-310. The DNA fragments encoding full length and a part of the human GnT-IV protein (Figure 33) lacking the TM domain are PCR amplified from the cDNA library using forward 5'-AATGAGATGAGGCTCCGCAATGGAACCTG-3' (SEQ ID NO. 17), 5'-CTGATTGCTTATCAACGAGAATTCCT- TG-3' (SEQ ID NO. 18), and reverse 5'-TGTTGGTTTCTCAGATGATCAGTTGGTG-3' (SEQ ID NO. 19) primers, respectively. The resulting PCR products are cloned and sequenced.

Similarly, genes encoding GnT-V protein have been isolated from several mammalian species, including mouse. (See, e.g., Alvarez, K. et al. *Glycobiology* 12 (7), 389-394 (2002)). The DNA fragments encoding full length and a part of the mouse GnT-V protein (Figure 34) lacking the TM domain are PCR amplified from the cDNA library using forward 5'-AGAGAGAGATGGCTTTCTTTCTCCCTGG-3' (SEQ ID NO. 20), 5'-AAATCAAGTGGATGAAGGACATGTGGC-3' (SEQ ID NO. 21), and reverse 5'-AGCGATGCTATAGGCAGTCTTTGCAGAG-3' (SEQ ID NO. 22) primers, respectively. The resulting PCR products are cloned and sequenced.--